

Appendix L-2

Discordant Substances for GHS Toxicity Category Predictions Using the 3T3 and NHK NRU Test Methods and Associated Regressions

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L.2 **Discordant Substances for GHS Toxicity Category Predictions Using the 3T3 and NHK NRU Test Methods and Associated Regressions**

This appendix provides a more detailed discussion of the discordant substances identified for the GHS acute oral toxicity category predictions using the NRU test methods and the regressions evaluated in **Section 6.3**.

L.2.1 Discordant Substances for Prediction of Toxicity Category by the 3T3 and NHK NRU Test Methods and the RC Millimole Regression

Table L2-1 identifies the discordant substances for which the *in vitro* predicted GHS toxicity category (using the 3T3 and NHK NRU test methods with the RC millimole regression) did not match the GHS toxicity category assigned based on the reference rodent LD₅₀ data. For the 3T3 NRU test method, the toxicity category was underpredicted for 20 (56%) and overpredicted for 14 (43%) of the 34 discordant substances. Of the 14 substances for which toxicity was underpredicted,

- 8 (57%) were underpredicted by one toxicity category
- 2 (14%) were underpredicted by two toxicity categories
- 4 (29%) were underpredicted by three toxicity categories.

For the 20 substances for which toxicity was overpredicted,

- 14 (70%) were overpredicted by one toxicity category
- 6 (30%) were overpredicted by two toxicity categories.

For the NHK NRU test method, toxicity was underpredicted for 12 (54%) and overpredicted for 22 (46%) of the 34 discordant substances. Of the 12 substances for which toxicity was underpredicted,

- 6 (50%) were underpredicted by one toxicity category
- 3 (25%) were underpredicted by two toxicity categories
- 2 (17%) were underpredicted by three toxicity categories
- 1 (8%) was underpredicted by four toxicity categories

For the 22 substances for which toxicity was overpredicted,

- 16 (73%) were overpredicted by one toxicity category
- 6 (27%) were overpredicted by two toxicity categories

The fact that there were more substances for which toxicity was overpredicted is a result of the removal of substances with specific mechanisms of toxicity that were not expected to be active in the 3T3 and NHK cell cultures. The toxicity for most of these substances would have been underpredicted. **Figure 3-1** shows that most of the 58 selected RC chemicals are below the RC regression line. Thus, the RC would predict lower toxicity (i.e., a higher LD₅₀) for most of these chemicals.

Table L2-1 Discordant Substances¹ for the Prediction of GHS² Toxicity Categories by the 3T3 and NHK NRU Test Methods and the RC Millimole Regression

Rodent GHS Toxicity Category ³ (mg/kg)	3T3 NRU Test Method		NHK NRU Test Method	
	Toxicity Overpredicted	Toxicity Underpredicted	Toxicity Overpredicted	Toxicity Underpredicted
LD ₅₀ < 5		Aminopterin (1) Busulfan (3) Cycloheximide (1) Mercury chloride (2) Phenylthiourea (3) Sodium selenate (3) Triethylenemelamine (1)		Aminopterin (4) Busulfan (3) Cycloheximide (1) Mercury chloride (2) Phenylthiourea (3) Sodium selenate (2) Triethylenemelamine (2)
5 < LD ₅₀ ≤ 50		Arsenic trioxide (1) Digoxin (3) Sodium arsenite (1) Sodium dichromate dihydrate (1) Thallium sulfate (2)		Arsenic trioxide (1) Sodium dichromate dihydrate (1) Thallium sulfate (1)
50 < LD ₅₀ ≤ 300		Cupric sulfate pentahydrate (1) Sodium oxalate (1)	Hexachlorophene (1)	Cupric sulfate pentahydrate (1) Sodium oxalate (1)
300 < LD ₅₀ ≤ 2000				
2000 < LD ₅₀ ≤ 5000	Acetaminophen (1) Acetonitrile (1) Boric acid (1) Chloramphenicol (1) Citric acid (1) Dimethylformamide (1) Lactic acid (1) Potassium chloride (1) Sodium chloride (1) Trichloroacetic acid (1) Xylene (1)		Acetaminophen (1) Acetonitrile (1) Boric acid (1) Chloramphenicol (1) Citric acid (1) Lactic acid (1) Potassium chloride (1) Sodium chloride (1) Trichloroacetic acid (1) Xylene (1)	
LD ₅₀ > 5000	5-Aminosalicylic acid (2) Dibutyl phthalate (2) Diethyl phthalate (2) Ethanol (2) Ethylene glycol (1)		5-Aminosalicylic acid (2) Dibutyl phthalate (2) Diethyl phthalate (2) Ethanol (1) Ethylene glycol (1)	

Rodent GHS Toxicity Category ³ (mg/kg)	3T3 NRU Test Method		NHK NRU Test Method	
	Toxicity Overpredicted	Toxicity Underpredicted	Toxicity Overpredicted	Toxicity Underpredicted
	Glycerol (1) 2-Propanol (2) Sodium hypochlorite (2) 1,1,1-Trichloroethane (1)		Gibberellic acid (1) Glycerol (1) Methanol (2) 2-Propanol (2) Sodium hypochlorite (2) 1,1,1-Trichloroethane (1)	

¹Substances for which the *in vitro* predicted GHS toxicity category was different from the GHS toxicity category assigned to the substance based on reference rodent LD₅₀ data. Numbers in parentheses indicate the number of categories different. Three substances were excluded because no rat LD₅₀ was identified: epinephrine bitartrate, colchicine, and propylparaben. Carbon tetrachloride was excluded from the 3T3 and NHK NRU analyses because no laboratory attained sufficient toxicity for the calculation of an IC₅₀. Methanol was excluded from the 3T3 analysis because no laboratory attained sufficient toxicity for the calculation of an IC₅₀. The 21 substances in **Table 6-3** were excluded based on their mechanisms of action.

²GHS-Globally Harmonized System of Classification and Labelling of Chemicals with LD₅₀ in mg/kg (UN 2005). The RC millimole regression is $\log LD_{50} (\text{mmol/kg}) = \log IC_{50} (\text{mM}) \times 0.435 + 0.625$.

³Reference rodent LD₅₀ values in from **Table 3-2**.

L.2.2 Discordant Substances for Prediction of Toxicity Category by the 3T3 and NHK NRU Test Methods and the RC Rat-Only Weight Regression

Table L2-2 shows the discordant substances for which the *in vitro* predicted GHS toxicity category (using the 3T3 and NHK NRU test methods with the RC rat-only weight regression) did not match that based on the reference rodent LD₅₀ data. The two *in vitro* NRU cytotoxicity test methods over- and under-predicted the GHS toxicity category for a similar number of substances. For the 3T3 NRU test method, the GHS toxicity category of 19 (63%) of 30 discordant substances was overpredicted, with:

- 13 (68%) overpredicted by one GHS toxicity category
- 6 (32%) overpredicted by two GHS toxicity categories

The toxicity of fewer substances (11; 37%) was underpredicted by this test method, with:

- 7 (64%) underpredicted by one GHS toxicity category
- 4 (36%) underpredicted by two GHS toxicity categories

For the NHK NRU test method, the GHS toxicity category of 22 (67%) of the 33 discordant substances was overpredicted. Of these,

- 15 (68%) were overpredicted by one GHS toxicity category
- 7 (31%) were overpredicted by two GHS toxicity categories

For this assay, the toxicity of 11 (33%) of the discordant substances was underpredicted, with

- 6 (55%) underpredicted by one GHS toxicity category
- 4 (36%) underpredicted by two GHS toxicity categories
- 1 (9%) underpredicted by three toxicity categories.

Phenylthiourea, the substance for which the GHS toxicity category was underpredicted by three toxicity categories, was in the most severe GHS toxicity category (i.e., LD₅₀ < 5 mg/kg).

Table L2-2 Discordant substances¹ for RC Rat-Only Weight Regression Prediction of GHS Toxicity Categories² by the 3T3 and NHK NRU Test Methods

Rodent GHS Toxicity Category ³ (mg/kg)	3T3 NRU Test Method		NHK NRU Test Method	
	Toxicity Overpredicted	Toxicity Underpredicted	Toxicity Overpredicted	Toxicity Underpredicted
LD ₅₀ < 5		Cycloheximide (1) Phenylthiourea (2) Sodium selenate (2) Triethylenemelamine (1)		Cycloheximide (1) Phenylthiourea (3) Sodium selenate (2) Triethylenemelamine (2)
5 < LD ₅₀ ≤ 50		Arsenic trioxide (1) Busulfan (2) Digoxin (2) Mercury chloride (1) Thallium sulfate (1) Sodium arsenite (1)		Aminopterin (2) Arsenic trioxide (1) Busulfan (2) Mercury chloride (1) Sodium arsenite (1) Thallium Sulfate (1)
50 < LD ₅₀ ≤ 300		Sodium fluoride (1)	Hexachlorophene (1)	Sodium fluoride (1)
300 < LD ₅₀ ≤ 2000	Propranolol (1) Triphenyltin hydroxide (2)		Triphenyltin hydroxide (2)	
2000 < LD ₅₀ ≤ 5000	Acetaminophen (1) 5-Aminosalicylic acid (1) Boric acid (1) Chloramphenicol (1) Xylene (1)		5-Aminosalicylic acid (1) Chloramphenicol (1) Boric acid (1) Xylene (1) Acetaminophen (1) Lactic acid (1) Sodium chloride (1) Potassium chloride (1)	
LD ₅₀ > 5000	Citric acid (2) Diethyl phthalate (2) Dibutyl phthalate (2) Dimethylformamide (1) Ethanol (1) Ethylene glycol (1) Gibberellic acid (1) Glycerol (1) 2-Propanol (1)		Citric acid (2) Dibutyl phthalate (2) Diethyl phthalate (2) Dimethylformamide (1) Ethanol (1) Gibberellic Acid (1) Glycerol (1) Methanol (2) 2-Propanol (1)	

Rodent GHS Toxicity Category ³ (mg/kg)	3T3 NRU Test Method		NHK NRU Test Method	
	Toxicity Overpredicted	Toxicity Underpredicted	Toxicity Overpredicted	Toxicity Underpredicted
	Sodium hypochlorite (2) Trichloroacetic acid (2) 1,1,1-Trichloroethane (1)		Sodium hypochlorite (2) Trichloroacetic acid (2) 1,1,1-Trichloroethane (1)	

¹Substances for which the *in vitro* predicted GHS toxicity category was different from that based on the reference rodent LD₅₀ data. Numbers in parentheses indicate the number of categories different. Three substances were excluded because no rat LD₅₀ was identified: epinephrine bitartrate, colchicine, and propylparaben. Carbon tetrachloride was excluded from the 3T3 and NHK NRU analyses because no laboratory attained sufficient toxicity for the calculation of an IC₅₀. Methanol was excluded from the 3T3 NRU analysis because no laboratory attained sufficient toxicity for the calculation of an IC₅₀. The 21 substances in **Table 6-3** were excluded based on their mechanisms of action.

²GHS-Globally Harmonized System of Classification and Labelling of Chemicals with LD₅₀ in mg/kg (UN 2005). The RC rat-only weight regression is $\log LD_{50} \text{ (mg/kg)} = \log IC_{50} \text{ (}\mu\text{g/mL)} \times 0.372 + 2.024$.

³Reference rodent LD₅₀ values from **Table 4-2**.

L.2.3 Discordant Substances for the Prediction of Toxicity Category by the 3T3 and NHK NRU Test Methods and the RC Rat-Only Weight Regression Excluding Substances with Specific Mechanisms of Toxicity

Table L2-3 shows the discordant substances for which the *in vitro* NRU predicted toxicity category (using the 3T3 and NHK NRU test methods with the RC rat-only weight regression excluding substances with specific mechanisms of toxicity) did not match that based on the reference rodent LD₅₀ data. The NHK NRU test method had four more discordant substances than the corresponding assay using 3T3 cells when the IC₅₀ results were applied to the RC rat-only weight regression, after excluding substances with specific mechanisms of toxicity. For the 3T3 NRU test method, the GHS toxicity category of 19 (63%) of 30 discordant substances was overpredicted, with

- 13 (68%) overpredicted by one toxicity category
- 6 (32%) overpredicted by two toxicity categories

The toxicity of 11 (37%) of 30 discordant substances was underpredicted by the 3T3 NRU test method, with

- 7 (64%) underpredicted by one category
- 4 (36%) underpredicted by two toxicity categories

For the NHK NRU test method, the toxicity of 22 (65%) of 34 discordant substances was overpredicted, with

- 15 (68%) overpredicted by one category
- 7 (32%) overpredicted by two toxicity categories

Also, for this test method, the toxicity of 12 (35%) of 34 discordant substances was underpredicted, with

- 6 (50%) underpredicted by one toxicity category
- 4 (33%) underpredicted by two toxicity categories
- 2 (17%) underpredicted by three toxicity categories

Both substances for which toxicity was underpredicted by three toxicity categories were in the most severe GHS toxicity category (i.e., LD₅₀ < 5 mg/kg).

Table L2-3 Discordant Substances¹ for RC Rat-Only Weight Regression Excluding Chemicals with Specific Mechanisms of Toxicity Prediction of GHS Toxicity Categories² by the 3T3 and NHK NRU Test Methods

Rodent GHS Toxicity Category ³ (mg/kg)	3T3 NRU Test Method		NHK NRU Test Method	
	Toxicity Overpredicted	Toxicity Underpredicted	Toxicity Overpredicted	Toxicity Underpredicted
LD ₅₀ < 5		Cycloheximide (1) Phenylthiourea (2) Sodium selenate (2) Triethylenemelamine (1)		Cycloheximide (1) Disulfoton (3) Phenylthiourea (3) Sodium selenate (2) Triethylenemelamine (2)
5 < LD ₅₀ ≤ 50		Arsenic trioxide (1) Busulfan (2) Digoxin (2) Mercury chloride (1) Thallium sulfate (1) Sodium arsenite (1)		Aminopterin (2) Arsenic trioxide (1) Busulfan (2) Mercury chloride (1) Sodium arsenite (1) Thallium sulfate (1)
50 < LD ₅₀ ≤ 300		Sodium fluoride (1)	Hexachlorophene (1)	Sodium fluoride (1)
300 < LD ₅₀ ≤ 2000	Propranolol (1) Triphenyltin hydroxide (2)		Triphenyltin hydroxide (2)	
2000 < LD ₅₀ ≤ 5000	Acetaminophen (1) 5-Aminosalicylic acid (1) Boric acid (1) Chloramphenicol (1) Xylene (1)		5-Aminosalicylic acid (1) Chloramphenicol (1) Boric acid (1) Xylene (1) Acetaminophen (1) Lactic acid (1) Sodium chloride (1) Potassium chloride (1)	
LD ₅₀ > 5000	Citric acid (2) Diethyl phthalate (2) Dibutyl phthalate (2) Dimethylformamide (1) Ethanol (1) Ethylene glycol (1) Gibberellic acid (1)		Citric acid (2) Dibutyl phthalate (2) Diethyl phthalate (2) Dimethylformamide (1) Ethanol (1) Gibberellic acid (1) Glycerol (1)	

Rodent GHS Toxicity Category ³ (mg/kg)	3T3 NRU Test Method		NHK NRU Test Method	
	Toxicity Overpredicted	Toxicity Underpredicted	Toxicity Overpredicted	Toxicity Underpredicted
	Glycerol (1) 2-Propanol (1) Sodium hypochlorite (2) Trichloroacetic acid (2) 1,1,1-Trichloroethane (1)		Methanol (2) 2-Propanol (1) Sodium hypochlorite (2) Trichloroacetic acid (2) 1,1,1-Trichloroethane (1)	

¹Substances for which the *in vitro* predicted GHS toxicity category was different from that based on the reference rodent LD₅₀ data. Numbers in parentheses indicate the number of categories different. Three substances were excluded because no rat LD₅₀ was identified: epinephrine bitartrate, colchicine, and propylparaben. Carbon tetrachloride was excluded from the 3T3 and NHK NRU analyses because no laboratory attained sufficient toxicity for the calculation of an IC₅₀. Methanol was excluded from the 3T3 NRU analysis because no laboratory attained sufficient toxicity for the calculation of an IC₅₀. The 21 substances in **Table 6-3** were excluded based on their mechanisms of action.

²GHS-Globally Harmonized System of Classification and Labelling of Chemicals with LD₅₀ in mg/kg (UN 2005). The RC rat minus specific mechanisms regression is $\log \text{LD}_{50} (\text{mg/kg}) = \log \text{IC}_{50} (\mu\text{g/mL}) \times 0.357 + 2.194$.

³Reference rodent LD₅₀ values from **Table 4-2**.

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